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The effect of using medicinal plant extracts upon the health of bee colonies

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Abstract

The productive potential of honeybee colonies is dependent on their state of health. The use of medicinal plant extracts in the end-of-winter protein-energy feed provided to bee colonies can furnish a method both for the improvement of bee health and for early mobilisation of colony numbers, with a major positive impact on honey yield. The feeding of bee colonies with protein-enriched candy in which extracts of garlic (Allium sativum), Echinaceea purpurea, Ganoderma lucidum and sage (Salvia officinalis) extracts had been incorporated has been shown both to stimulate queen brood laying and to lead to a reduction in the numbers of bee intestinal bacteria.

Keywords: honeybee, garlic extract, *Echinaceea* extract, *Ganoderma* extract, sage extract

1. Introduction

Medicinal plants contain significant quantities of antimicrobial substances (LEWIS & ASUBEL [1]), which can be used as an alternative to antibiotics in prophylactic treatment of some bee diseases (FLESAR & al. [2], MĂRGHITAȘ & al. [3]). Extensive research has been done on the effectiveness of such utilisation of plant extracts arising from their antibacterial, antiviral, antifungal, and anti-inflammatory properties (MĂRGHITAȘ & al. [3], PĂTRUICĂ & al. [4], PĂTRUICĂ & al. [5], MĂRGHITAȘ & al. [6], PĂTRUICĂ & al. [7], ZAROG & BASSIOUNY [8], QAYYOUM & al. [9]).

Research using both *Apis mellifera* and *Apis carnica* bee colonies infested with *Varroa jacobsoni* and *Varroa destructor* parasites has demonstrated the value of medicinal plants in the eradication of diseases (ZAROG & BASSIOUNY [8], QAYYOUM & al. [9], MAHMOOD & al. [10]).

The antimicrobial effect of bay leaves (*Laurus nobilis*) on the bacterium *Paenibacillus larvae* that causes American Foulbrood disease was shown by (MAGGI & al. [11]). These authors also noted the anti-parasite effect of bay leaves on *Varroa destructor* and *Nosema ceranae* parasites. Similar results had been obtained by (PORINI & al., [12] and DAMIANI & al. [13]).

Results which highlight antimicrobial activity on the *Paenibacillus larvae* bacterium were obtained using extracts of basil (*Ocimum basilicum*), nettle (*Urtica dioica*), thyme (Satureja hortensis) and yarrow (*Achillea millefolium*) (MĂRGHITAȘ & al. [6]), DAMIANI & al. [13], POHORECKA [14]).

2. Materials and Methods

Research was carried out using 50 colonies of *Apis mellifera carpatica* housed in sectional hives. The colonies were divided into five equal experimental treatment groups matched for strength and queen age. On February 5 2015 each colony was fed 1 kg of protein-enriched bee candy supplemented with a medicinal plant extract (garlic (*Allium sativum*), *Echinaceea*, *Ganoderma* or sage (*Salvia officinalis*). Concomitently, a sample of ten bees from each colony was taken, using sterile collection tubes, and subjected to lab microbiological examination.

The protein-enriched candy was prepared from honey, sugar and pollen to have a crude protein content of 15%. This was placed inside the hive above the frames. 28 days after the candy had been given (March 5 2015) the area of comb occupied by brood cells (both capped and uncapped) was measured for each colony in order to assess the effect on the egg-laying activity of the queen of the plant extracts administered. 100 section Netz frame grids were used. At the same time a sample of a further 10 worker bees from each colony was taken, with these being transported within a short time (30 mins) to the lab for microbiological examination to determine the effect of the administration of plant extracts on the intestinal microflora.

Under laboratory conditions each bee intestine was transferred using a fine brush into sterile distilled water using standard aseptic technique, with a separate tube being used for each colony. Serial dilution plating was performed using 10^{-1}, 10^{-2} and 10^{-3}, dilutions with 10 nutrient agar plates being seeded from each dilution (PĂTRUICĂ & MOȚ [15]). Following Petri dish incubation (37^0C, 24 hours) the 10^{-3} dilution plates were found most useful for colony counting, having a sufficient density of distinct non-confluent colonies. Gram stained smears from each sample were microscopically examined. Colony counting was realised using a Nitech LKB 2002 apparatus.

Estimation of number of viable bacteria was carried out twice for each treatment group. Statistical analysis both of colony development and of worker bee intestinal microbial load following plant extract use was carried out using IBM SPSS Statistics ANOVA software package version 19. This provided comparisons of brood cell area coverage (capped and uncapped) and of intestinal microbial populations between treatment and control groups.

3. Results and Conclusions

End of winter feeding of bee colonies with protein-enriched candy supplemented with plant extracts had the effect of stimulating queen oviposition. Best results were obtained from colonies given *Ganoderma* extract (EG_3), which showed a significantly higher number ($P<0.01$) of brood cells as compared with the control. Significant results ($P<0.05$) for brood cell occupation were also achieved by colonies given *Echinaceea* extract (EG_2).

Addition of garlic extract (1g/kg candy) or of sage extract (1g/kg) did not significantly increase the number of occupied brood cells in the colonies studied (EG_1 and EG_4) – table 1. It was also noticed that garlic doped candy (EG_1) was less consumed, possibly due to its resultant smell.

Addition of the plant extracts studied to the candy both increased the number of brood cells and reduced the number of bacteria in worker bee intestines. *Bacillus spp.*, *Pseudomonas spp.* and *Staphylococcus spp.* had been identified in the bee intestines at the start of the experiment.

Garlic extract (EG_1) had the effect of significantly reducing ($P<0.05$) the number of bacteria in the small intestine 28 days after the candy was given. (1338 cm^{-3} at the start and 1034 cm^{-3} after 28 days – see table 1.) Significant results were also observed for colonies fed *Echinaceea* extract (EG_2) (1788 cm^{-3} initially and 1485 after 28 days).

Although colonies fed *Ganoderma* extract (EG_3) and sage extract (EG_4) showed a fall in intestinal bacterial population over the course of the experiment, the results were not statistically significant (table 1).

Table 1. Mean and standard deviation values for number of viable bacteria present in the intestines of bees given plant extracts (n=100).

Plant extracts				Without plant extracts			
At the start of the experiment period		At the end of the experiment period		At the start of the experiment period		At the end of the experiment period	
EG_1	1338±126	EG_1	$1034±132^{*}$	CG	1434±94	CG	$11478±105^{NS}$
EG_2	1788±106	EG_2	$1485±114^{*}$				
EG_3	1342±134	EG_3	$1207±98^{NS}$				
EG_4	1802±182	EG_4	$1596±103^{NS}$				

NS - not significant

$^{*}P<0.05$

** $P<0.01$

CG, control group; EG_1, garlic extract (1g) treatment group; EG_2, *Echinaceea* extract (1g) treatment group; EG_3, *Ganoderma* extract (1g) treatment group; EG_4, sage extract (1g) treatment group.

The early seasonal development of bee colonies is extremely important for their more effective exploitation of nectar-producing plants, which is positively correlated with higher apicultural product yields (PĂTRUICĂ & HUȚU [6]). Addition of plant extracts (garlic, *Ganoderma*, *Echinceea*, sage) to feed candy given to colonies had the effect of stimulating queens, as shown by an increase in brood comb coverage. This has also been observed by other researchers who have provided stimulating feeds of sugar syrup or candy to which biostimulators had been added (PĂTRUICĂ & al. [4]), PĂTRUICĂ & al. [5]), MĂRGHITAȘ & al. [6], PĂTRUICĂ & al. [7]), EREMIA & al. [17]).

The best colony dynamics were recorded for colonies fed candy doped with *Echinaceea* (EG_2) and *Ganoderma* (EG_3). After 28 days these colonies produced between 1685 and 2385 more brood cells than the control group. Although addition of garlic extract to the end-of-winter candy feed resulted in a slight fall in feed consumption it did have the effect of stimulating the egg-laying activity of the queen, with colonies in EG_1 producing 24.5% more brood cells than the control. At the same time these colonies showed a reduction of 22.7% in the bacterial population of worker bee small intestines (table 1). Researchers have also demonstrated the beneficial effect of garlic extract in controlling the *Nosema apis* parasite (BURA & al. [18]).

A reduction in worker bee small intestine bacterial population was observed in all experimental groups. Thus, in the 28 days from the administration of plant extract doped candy, the bacterial population diminished by 16.94% for the *Echinaceea* group, 10.05% for EG_3 (*Ganoderma* extract), and 11.43% for EG_4 (sage extract).

We may conclude that the use of the plant extracts studied as additions to end-of-winter stimulating or supplementary feeds for honeybees had the double effect of both stimulating the egg-laying activity of the queen and reduced the number of bacteria in worker bee intestine by ensuring the health of bee families. We can expect that this will result in an increased population of healthy worker bees able to effectively exploit the honey-yielding resources of their environment throughout the foraging season.

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References

1. K. LEWIS, F.M. AUSUBEL. Prospects for plant–derived antimicrobials. Nature Biotech, 24: 1504–1507 (2006).

2. J. FLESAR, J. HAVILIK, P. KLOUCEK, V. RADA, D. TITERA, M. BEDNAR, M. STROPNICKY, L. KOKOSKA. In vitro growth-inhibitory effect of plant-derived extracts and compounds against Paenibacillus larvae and their acute oral toxicity to adult honey bees. Vet. Microb, 145: 129-133 (2010).

3. L.A. MĂRGHITAȘ, D. DEZMIREAN, F. CHIRILĂ, F. NICODIM, O. BOBIȘ. Antibacterial activity of different plant extract and phenolic phytochemicals tested on Paenibacillus Larvae bacteria. Sci. Paper Animal Sci. & Biotech, 44(2): 94-99 (2011).

4. S. PĂTRUICĂ, M. BURA, I. BĂNĂTEAN DUNEA, I. POPESCU, E. SIMIZ, P. SCHIOPESCU. Research on the influence of some apiary biostimulators on the development of bee colonies salping in the autumn season. Sci. Paper Animal Sci. & Biotech, 39: 117-123 (2006).

5. S. PĂTRUICĂ, M. BURA, I. BĂNĂȚEAN DUNEA, P. SCHIOPESCU. Research on the influence of onion and brier extracts on bee families development. In: Proceedings of the 1st International Conference Apiculture from science to agribusiness and apitherapy. Cluj-Napoca, România, pp 157-167 (2007).

6. L.A. MĂRGHITAȘ, O. BOBIȘ, M. TOFALVI, The effect of plant supplements on development of artificially weaken bee families. Sci. Paper Animal Sci. & Biotech, 43(1): 402-406 (2010).

7. S. PĂTRUICĂ, I. BĂNĂȚEAN- DUNEA, A. JIVAN, A. JIVAN, A. STROE. Research on the influence of apiary biostimulators on bee families development in spring. Sci. Paper Animal Sci. & Biotech, 44(2): 267-271 (2011).

8. A.A. AL-ZAROG, A.M. EL-BASSIOUNY. Influence of some plant extracts on Varroa mite and performance of honey bee Apis mellifera colonies, E. Acad. J. Biol. Sci. 5(2): 15-20 (2013).

9. M.A. QAYYOUM, B.S. KHAN, M.H. BASHIR. Efficacy of plant extracts against honey bee mite, Varroa destructor (Acari:Varroidae). World J. Zoology, 8(2):212-216 (2013).

10. R. MAHMOOD, S. ASAD, S. RAJA, A. MOSHIN, E.S. WAGCHOURE, G. SARWAR, N. ISLAM, W. AHMAD. Control of Varroa destructor (Acari:Varroidae) in *Apis mellifera* (Hymenoptera : Apidae) by using plant oils and extract. Pakistan J. Zoology, 46(3): 609-615 (2014).

11. M.D. MAGGI, J.A. MARCANGELI, M.J. EQUARAS. Laurel leaf extracts for honeybee pest and disease management: antimicrobial, microsporicidal and acaricidal activity. Parasitol. Res, 103(2): 701-709 (2014).

12. M.P. PORRINI, N. FERNANDEZ, P. GARRIDO, S. MEDICI, L.B. GENDE, S.C. MEDICI, M.J. EGUARAS. In vivo evaluation of antiparasitic activity of plant extracts on Nosema ceranae (Microsporidia). Apidol, 42 (6):700-707 (2011).

13. N. DAMIANI, N.J. FERNANDEZ, M.P. PORINI, M.P. GENDE, E. ALVAREZ, F. BUFFA, C. BRASESCO, K. KUZYŠINOVÁ, D. MUDROŇOVÁ, J. TOPORČÁK, R. NEMCOVÁ, L. MOLNÁR, A. MAĎARI, S. VANÍKOVÁ, M. KOŽÁR. Testing of inhibition activity of essential oils against Paenibacillus larvae– the causative agent of American foulbrood. Acta Veter. Brno, 83: 9–12 (2014).

14. K. POHORECKA. Effect of standardized plant herb extracts on general condition of the honeybee (Apis mellifera l.). Bulletin of Veterinary Institute in Pulawy, 48: 415-419 (2004).

15. S. PĂTRUICĂ, D. MOȚ. The effect of using prebiotic and probiotic products on intestinal micro-flora of the honeybee (Apis mellifera carpatica). B. Entomol. Res, 102 (6): 619-623 (2012).

16. S. PĂTRUICĂ, I. HUȚU. Economic benefits of using prebiotic and probiotic products as supplements in stimulation feeds administered to bee colonies. Turk. J. Vet. Med. & Anim. Sci, 37 (3):259-263 (2013).

17. N. EREMIA, A. ZAGAREANU, T. MARDARI, S. MODVALA. Stimulation of resistance of bee families during wintering. Sci. Paper Animal Sci. & Biotech, 46(2): 268-271 (2013).

18. M. BURA, S. PĂTRUICĂ, D. ONIȚA CHIȘ. Researches on the influence of apiary biostimulators on bee families brood's health and development during spring season. In: Proceedings of the 1st International Simposyum organized by Banat University of Agricultural Sciences and Veterinary Medicine Timișoara. România pp 88-99 (2004).